



(12) **EUROPEAN PATENT APPLICATION**

(88) Date of publication A3:  
**28.01.2004 Bulletin 2004/05**

(51) Int Cl.7: **C12Q 1/68, C12N 15/10,  
G01N 33/50**

(43) Date of publication A2:  
**07.08.2002 Bulletin 2002/32**

(21) Application number: **02250681.0**

(22) Date of filing: **31.01.2002**

(84) Designated Contracting States:  
**AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU  
MC NL PT SE TR**  
Designated Extension States:  
**AL LT LV MK RO SI**

(30) Priority: **31.01.2001 US 265805 P  
26.10.2001 US 339260 P**

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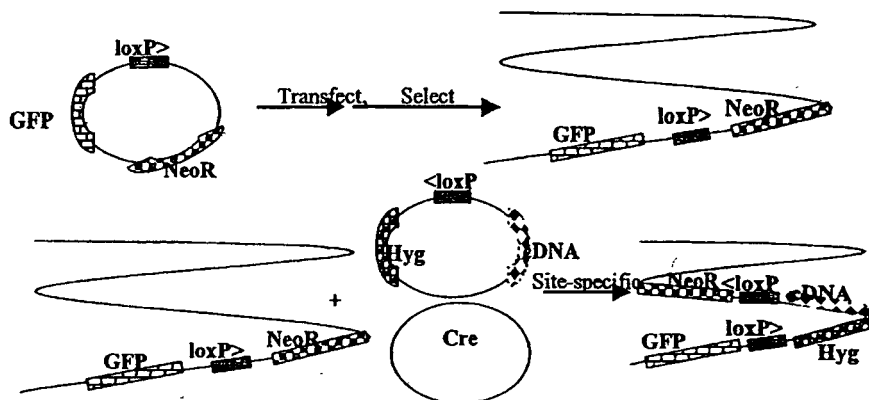
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(54) **Use of post-transcriptional gene silencing for identifying nucleic acid sequences that modulate the function of a cell**

(57) Described herein are methods for identifying nucleic acid sequences that modulate the function of a cell, the expression of a gene in a cell, or the biological

activity of a target polypeptide in a cell. The methods involve the use of double stranded RNA expression libraries, double stranded RNA molecules, and post-transcriptional gene silencing techniques.

**Fig. 1**





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# EUROPEAN SEARCH REPORT

Application Number  
EP 02 25 0681

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	<p>UI-TEI K ET AL: "Sensitive assay of RNA interference in Drosophila and Chinese hamster cultured cells using firefly luciferase gene as target"</p> <p>FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 479, no. 3, 18 August 2000 (2000-08-18), pages 79-82, XP004337469</p> <p>ISSN: 0014-5793</p> <p>* abstract *</p> <p>* page 81, column 1, paragraph 2 - page 82, column 1, paragraph 4; figures 1,3 *</p> <p>---</p>	1-39	<p>C12Q1/68</p> <p>C12N15/10</p> <p>G01N33/50</p>
X	<p>WIANNY F ET AL: "Specific interference with gene function by double-stranded RNA in early mouse development"</p> <p>NATURE CELL BIOLOGY, MACMILLAN PUBLISHERS, GB, vol. 2, no. 2, February 2000 (2000-02), pages 70-75, XP002138445</p> <p>ISSN: 1465-7392</p> <p>* abstract *</p> <p>* page 73, column 2, paragraph 1 - page 74, column 1, paragraph 3; figures 2,3,5,6 *</p> <p>---</p> <p style="text-align: center;">-/--</p>	23-39	<p>TECHNICAL FIELDS SEARCHED (Int.Cl.7)</p> <p>C12N</p> <p>C12Q</p>
The present search report has been drawn up for all claims			
Place of search		Date of completion of the search	Examiner
MUNICH		9 December 2003	Bradbrook, D
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X: particularly relevant if taken alone</p> <p>Y: particularly relevant if combined with another document of the same category</p> <p>A: technological background</p> <p>O: non-written disclosure</p> <p>P: intermediate document</p> <p>T: theory or principle underlying the invention</p> <p>E: earlier patent document, but published on, or after the filing date</p> <p>D: document cited in the application</p> <p>L: document cited for other reasons</p> <p>§: member of the same patent family, corresponding document</p>			

EPO FORM 1503 (03.02.99) (P4/C01)



European Patent  
Office

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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	<p>SVOBODA P ET AL: "SELECTIVE REDUCTION OF DORMANT MATERNAL MRNAS IN MOUSE OOCYTES BY RNA INTERFERENCE"</p> <p>DEVELOPMENT, COMPANY OF BIOLOGISTS, CAMBRIDGE,, GB,</p> <p>vol. 127, no. 127, 2000, pages 4147-4156, XP001064763</p> <p>ISSN: 0950-1991</p> <p>* abstract *</p> <p>* page 4152, column 1, paragraph 3 - page 4153, column 2, paragraph 1 *</p> <p>* page 4154, column 2, paragraphs 1,2; figures 2,3,5,6 *</p> <p>---</p>	23-39	<p>TECHNICAL FIELDS SEARCHED (Int.Cl.7)</p>
X	<p>WARGELIUS A ET AL: "DOUBLE-STRANDED RNA INDUCES SPECIFIC DEVELOPMENTAL DEFECTS IN ZEBRAFISH EMBRYOS"</p> <p>BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, ACADEMIC PRESS INC. ORLANDO, FL, US,</p> <p>vol. 263, no. 1,</p> <p>16 September 1999 (1999-09-16), pages 156-161, XP000872838</p> <p>ISSN: 0006-291X</p> <p>* abstract; figure 2; table 1 *</p> <p>---</p>	23-39	
X	<p>ELBASHIR SAYDA M ET AL: "RNA interference is mediated by 21- and 22-nucleotide RNAs"</p> <p>GENES AND DEVELOPMENT, COLD SPRING HARBOR LABORATORY PRESS, NEW YORK, US,</p> <p>vol. 15, no. 2,</p> <p>15 January 2001 (2001-01-15), pages 188-200, XP002204651</p> <p>ISSN: 0890-9369</p> <p>* abstract; figures 1,5 *</p> <p>---</p> <p>-/--</p>	23-39	
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EPO FORM 1503 (03.02.02) (P04001)



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Office

# EUROPEAN SEARCH REPORT

Application Number  
EP 02 25 0681

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	WO 00 63364 A (AMERICAN HOME PROD ;PACHUK CATHERINE (US); SATISHCHANDRAN C (US)) 26 October 2000 (2000-10-26) * page 8, line 6 - page 9, line 28 * * page 17, line 12 - page 20, line 16; examples 1-4 *	1-39	
X	WO 99 32619 A (CARNEGIE INST OF WASHINGTON ;MONTGOMERY MARY K (US); FIRE ANDREW ()) 1 July 1999 (1999-07-01) * page 6, line 1 - page 8, line 2 * * page 9, line 15 - page 20, line 15; claims 1,21 *	1-39	
A	BOSHER J M ET AL: "RNA INTERFERENCE: GENETIC WAND AND GENETIC WATCHDOG" NATURE CELL BIOLOGY, MACMILLAN PUBLISHERS, GB, vol. 2, no. 2, February 2000 (2000-02), pages E31-E36, XP001106960 ISSN: 1465-7392 * the whole document *	1-39	
P,X	ELBASHIR SAYDA M ET AL: "Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 411, no. 6836, 24 May 2001 (2001-05-24), pages 494-498, XP002206451 ISSN: 0028-0836 * the whole document *	23-39	
P,A	WO 01 75164 A (MAX PLANCK GESELLSCHAFT ;TUSCHL THOMAS (DE); MASSACHUSETTS INST TE) 11 October 2001 (2001-10-11) * the whole document *		
-/--			
The present search report has been drawn up for all claims			
Place of search MUNICH		Date of completion of the search 9 December 2003	Examiner Bradbrook, D
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons &amp; : member of the same patent family, corresponding document</p>			

EPO FORM 1503 (03.02.02) (P/MC01)



European Patent  
Office

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Application Number  
EP 02 25 0681

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
E	WO 02 44321 A (MAX PLANCK GESELLSCHAFT ;TUSCHL THOMAS (DE); ELBASHIR SAYDA (DE);) 6 June 2002 (2002-06-06) * the whole document * -----	1-39	
			TECHNICAL FIELDS SEARCHED (Int.Cl.7)
The present search report has been drawn up for all claims			
Place of search MUNICH		Date of completion of the search 9 December 2003	Examiner Bradbrook, D
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

EPO FORM 1503 03 82 (P04C01)

ANNEX TO THE EUROPEAN SEARCH REPORT  
ON EUROPEAN PATENT APPLICATION NO.

EP 02 25 0681

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09-12-2003

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0063364	A	26-10-2000	AU 4472100 A	02-11-2000
			BR 0009884 A	08-01-2002
			CA 2370628 A1	26-10-2000
			CN 1375004 T	16-10-2002
			EP 1171586 A2	16-01-2002
			JP 2002542263 T	10-12-2002
			WO 0063364 A2	26-10-2000
WO 9932619	A	01-07-1999	US 6506559 B1	14-01-2003
			AU 743798 B2	07-02-2002
			AU 1938099 A	12-07-1999
			CA 2311999 A1	01-07-1999
			EP 1042462 A1	11-10-2000
			JP 2002516062 T	04-06-2002
			WO 9932619 A1	01-07-1999
			US 2003056235 A1	20-03-2003
			US 2003051263 A1	13-03-2003
			US 2003055020 A1	20-03-2003
WO 0175164	A	11-10-2001	AU 3574402 A	11-06-2002
			AU 4962201 A	15-10-2001
			CA 2404890 A1	11-10-2001
			CA 2429814 A1	06-06-2002
			CZ 20031839 A3	15-10-2003
			WO 0244321 A2	06-06-2002
			EP 1309726 A2	14-05-2003
			HU 0302557 A2	28-10-2003
			JP 2003529374 T	07-10-2003
			NO 20032464 A	21-07-2003
			WO 0175164 A2	11-10-2001
			US 2003108923 A1	12-06-2003
			US 2002086356 A1	04-07-2002
WO 0244321	A	06-06-2002	AU 3574402 A	11-06-2002
			AU 4962201 A	15-10-2001
			CA 2404890 A1	11-10-2001
			CA 2429814 A1	06-06-2002
			CZ 20031839 A3	15-10-2003
			WO 0244321 A2	06-06-2002
			EP 1309726 A2	14-05-2003
			HU 0302557 A2	28-10-2003
			JP 2003529374 T	07-10-2003
			NO 20032464 A	21-07-2003
			WO 0175164 A2	11-10-2001
			US 2003108923 A1	12-06-2003
			US 2002086356 A1	04-07-2002

EPO FORM P0499

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